PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 6342PTWO-ca	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/053129	International filing date *(day/month/year)* 26.11.2004	Priority date *(day/month/year)* 27.11.2003
International Patent Classification (IPC) or national classification and IPC A61L27/42, A61L27/20, A61L27/38		
Applicant FIDIA ADVANCED BIOPOLYMERS S.R.L.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 8 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27.09.2005	13.03.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/053129

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-19 as originally filed

   Claims, Numbers

   1-60, 62-74 received on 01.11.2005 with letter of 25.10.2005

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-60,62-74
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-60,62-74
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-60,62-74
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V.

1 Reference is made to the following documents:

D1: WO 02/070030 A
D2: WO 93/20858 A
D3: BAKOS D ET AL: "Hydroxyapatite-collagen-hyaluronic acid composite" January 1999, BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, PAGE(S) 191-195 , ISSN: 0142-9612
D4: WO 95/01181 A

2 NOVELTY

2.1 D1 discloses a three-dimensional bilayer composite material comprising hyaluronic acid derivatives with a ceramic matrix that can also comprise active ingredients. For example, a cell-loaded HYAFF-11 sponge and porous calcium phosphate ceramic were assembled and joined together with fibrin glue to form a composite osteochondral graft (see D1, page 3, line 21 to page 9, line 3; page 11, line 8 to page 12, line 19; claims). The difference between D1 and the subject-matter of independent claim 1 of the present application is that unlike the bilayer composite disclosed in D1, the ceramic material and hyaluronic acid (HYA) derivative form together a single layer and they are not separated from each other. Also the second layer in the composite material disclosed in the subject-matter of independent claim 1 of the present application, may be fixed with fibrin glue to the inner matrix, is formed by a hyaluronic acid derivative.

2.2 D2 discloses a composite material paste for bone replacement comprising, e.g. HYAFF and hydroxyapatite (see D2, examples 38-40; claims). Since D2 discloses a paste which is prepared in situ it is clearly different from the composite material as disclosed in claim 1 of the present application.

2.3 D3 discloses a composite biomaterial made from hydroxyapatite and collagen conjugated with hyaluronic acid (see D3, abstract). The composite material is prepared with a method comprising a) HYA particles being gradually added to the solution of HYA sodium salt in deionised water and intensively mixed, b) preparing an aqueous dispersion

of very fine collagen fibers, c) mixing the two dispersions to form a complex precipitate, which is filtered and dried (see D3, page 192, paragraphs 2.1 and 2.2). Hence, the material obtained with this process is not a multilayer composite material as disclosed in the subject-matter of claim 1 of the present application.

2.4 D4 discloses a composite material which is a monolayer matrix comprising hyaluronic acid derivatives and matrix materials, e.g. hydroxylapatite (see D4, page 11, line 14-29; examples; claims). D4 does not disclose the multilayer composite material disclosed in the subject-matter of claim 1 of the present application.

2.5 Hence, the subject-matter of claims 1-60,62-74 is new in the sense of Article 33(2) PCT.

3 INVENTIVE STEP

3.1 The problem to be solved can be regarded as to provide a material to be used for bone tissue regeneration [especially in the field of spinal surgery and in particular for surgical operations involving the fusion of two vertebrate (see page 1, lines 1-23)].

3.2 D1, which may be considered as the most relevant prior art, discloses a three-dimensional bilayer composite material comprising hyaluronic acid derivatives with a ceramic matrix that can also comprise active ingredients. For example, a cell-loaded HYAFF-11 sponge and porous calcium phosphate ceramic were assembled and joined together with fibrin glue to form a composite osteochondral graft (see D1, page 3, line 21 to page 9, line 3; page 11, line 8 to page 12, line 19; claims).

3.3 The difference between D1 and the subject-matter of independent claim 1 of the present application is that unlike the bilayer composite disclosed in D1, the ceramic material and hyaluronic acid (HYA) derivative form together a single layer and they are not separated from each other. Also the second layer in the composite material disclosed in the subject-matter of independent claim 1 of the present application, may be fixed with fibrin glue to the inner matrix, is formed by a hyaluronic acid derivative.

3.4 The technical problem solved by D1 is different than the present application. D1

means to find a composite material to be used in an osteochondral graft containing both cartilage and a bone part which is achieved with a bilayer composite material whose first layer is a porous 3-D matrix constituted by a ceramic material and the second layer is formed by a porous 3-D matrix constituted by a hyaluronic acid derivative (see D3, page 7, lines 9-16; example 1; claim 1).

3.5 The skilled person faced with the problem of finding a composite material to be effectively used in spinal fusion could in no way infer that this technical problem could be easily overcome with the multilayer composite material as disclosed in the subject-matter of the present claims, from D1 solving a different technical problem and moreover with a different bilayer composite material.

3.6 Hence, the subject-matter of claims 1-60,62-74 can be considered inventive in the sense of Article 33(3) PCT.

NEW SET OF CLAIMS

1. A multilayer composite material comprising as the inner matrix a composite material comprising :

(i) hyaluronic acid and/or hyaluronic acid derivatives,

(ii) a matrix of demineralised bone and/or biocompatible and biodegradable ceramics and/or bone of autologous or allogenic or animal origin.

in association with at least one layer comprising a hyaluronic acid derivative.

2. The multilayer composite material according to claim 1 , wherein the hyaluronic acid in (i) is salified with organic or inorganic bases.

3. The multilayer composite material, according to anyone of claims 1 and 2, wherein said hyaluronic acid derivative in (i) is selected from the group consisting of:

A) esters of hyaluronic acid,

B) inner esters of hyaluronic acid with a percentage

C) amides of hyaluronic acid

D) O-sulphated derivatives of hyaluronic acid,

E) deacetylated derivatives of hyaluronic acid

F) percarboxylated derivatives of hyaluronic acid.

4. The multilayer composite material according to claim 3, wherein said hyaluronic acid ester is the benzyl ester.

5. The multilayer composite material according to claim 4 wherein the benzyl ester has a degree of esterification of from 50 to 100%.

6. The multilayer composite material according to claim 5, wherein the benzyl ester has a degree of esterification of from 75 to 100%.

7. The multilayer composite material as claimed in claim 3 wherein the hyaluronic acid inner esters have an esterification degree lower than 20%.

8. The multilayer composite material as claimed in claim 7, wherein the hyaluronic acid inner esters have an esterification degree comprised between 0.05 and 5%.

9. The multilayer composite material as claimed in claim 3 wherein the amidation degree of hyaluronic acid amides (C) is lower than or equal to 15%.

10. The multilayer composite material as claimed in claim 9, wherein the amidation

degree is comprised between 0,1 and 15%.

11. The multilayer composite material as claimed in claim in claim 3 wherein the deacetylated hyaluronic acid has a percentage of deacetylation lower than or equal to 30%.

12. The multilayer composite material as claimed in claim 3, wherein the percarboxylated hyaluronic acid (F) has a percarboxylation degree of between 0.1and 100%.

13. The multilayer composite material as claimed 12, wherein said percarboxylation degree is comprised between 25 and 75%.

14. The multilayer composite material according to anyone of claims 1-13, wherein the biocompatible and biodegradable ceramics is selected from the group consisting of hydroxyapatite and/or tribasic calcium phosphate and/or calcium sulphate.

15. The multilayer composite material according to anyone of claims 1-13, wherein the bone matrix is partially or completely demineralised.

16. The multilayer composite material according to anyone of claims 1-15 wherein the hyaluronic acid derivative has a molecular weight of between 200 and 750 kDs.

17. The multilayer composite material according to anyone 1-16 wherein the hyaluronic acid derivative is in a form selected from the group consisting of a non woven tissue, a sponge, a paste, granules, and powders.

18. The multilayer composite material according to any one of claims 1-17, wherein the layers are 2.

19. The multilayer composite material according to anyone of claims 1-18, wherein the layers are 3.

20. The multilayer composite material, according to anyone of claims 1-19, wherein said hyaluronic acid derivative contained in the layer(s) is selected from the group consisting of:

A) esters of hyaluronic acid,

B) inner esters of hyaluronic acid with a percentage

C) amides of hyaluronic acid

D) O-sulphated derivatives of hyaluronic acid,

E) deacetylated derivatives of hyaluronic acid

F) percarboxylated derivatives of hyaluronic acid

21. The multilayer composite material according to claim 20, wherein said hyaluronic acid ester is the benzyl ester.

22. The multilayer composite material according to claim 21, wherein the benzyl ester has a degree of esterification of from 50 to 100%.

23. The multilayer composite material according to 22, wherein the benzyl ester has a degree of esterification of from 75 to 100%.

24. The multilayer composite material according to claim 20, wherein the hyaluronic acid inner esters have an esterification degree lower than 20%.

25. The multilayer composite material according to claim 24, the hyaluronic acid inner esters have an comprised between 0.05 and 5%.

26. The multilayer composite material according to claim 20, wherein the amidation degree of hyaluronic acid amides (C) is lower than or equal to 15%.

27. The multilayer composite material according to claim 26, wherein the amidation degree is comprised between 0,1 and 15%.

28. The multilayer composite material according to claim 20, wherein the deacetylated hyaluronic acid has a percentage of deacetylation lower than or equal to 30%.

29. The multilayer composite material according to claim 20, wherein the percarboxylated hyaluronic acid (F) has a percarboxylation degree of between 0.1 and 100%.

30. The multilayer composite material according to claim 29, wherein said percarboxylation is comprised between 25 and 75%.

31. The multilayer composite material according to anyone of claims 1-30, wherein the hyaluronic acid derivatives comprised in the layer(s) are in the form selected from the group consisting of: non woven material, woven material, and compact, perforated porous or microporous membranes and films.

32.The multilayer composite material according to anyone of claims 1-31, wherein the inner matrix is in the form of a sponge consisting of the benzyl ester of hyaluronic acid with a percentage of esterification ranging between 70 and 100%, containing inside said sponge:

- bone granules or powders that are autologous and/or allogenic and/or of animal origin, or
- granules or other two- or three-dimensional structures constituted by biodegradable ceramics or, lastly,
- partially or completely demineralised bone matrix.

33. The multilayer composite material according to claim 1, subsequently coated throughout with HA and/or the derivatives thereof in the form of a thin film and/or sponge, to favour the entry, distribution and adhesion of the cells that will migrate once they have been loaded therein.

34. The multilayer composite materials according to claim 1, wherein the inner matrix is in the form of sponges formed by the inner esters of HA containing inside them:

- bone granules and/or powders of autologous and/or allogenic type and/or of animal origin,
- biodegradable ceramics or
- partially or completely demineralised bone matrix.

35. The multilayer composite materials according to claim 1, wherein the inner matrix is in the form granules, spheres, powders and/or two- and three-dimensional structures of various shapes and sizes consisting of biodegradable ceramics that are coated/incorporated in a layer of HA subsequently cross-linked to form its inner ester (ACP) which thus covers all the ceramic structures.

36. The multilayer composite materials according to claim 1, wherein the inner matrix is in the form of pastes and/or gels consisting of HA derivatives enclosing bone powders and/or granules that are autologous and/or allogenic and/or of animal origin, or granules or other two- or three-dimensional structures constituted by biodegradable ceramics or, lastly, pastes and/or gels containing demineralised bone matrix.

37. The multilayer composite materials according to of claim 1, wherein the inner matrix is in the form of fibres comprising the benzyl ester of HA with a percentage of esterification ranging between 50 and 100%, possibly associated with other natural polymers selected from collagen and cellulose and the derivatives thereof, or synthetic polymers selected from poly-lactic, polyglycolic and poly-

caprolactone acid, in association with demineralized bone matrix and hyaluronic acid.

38. The multilayer composite materials according to claim 37, wherein the matrix can be wetted with a solution of hyaluronic acid ester , to render it more compact with the layers between which it is sandwiched.

39. The multilayer composite materials according to anyone of claims 37 and 38, wherein said matrix consists of fibres of hyaluronic acid benzylester having an esterification degree of 75% in amounts ranging from 10 to 50% and demineralised bone matrix in amounts ranging from 50 to 90% and htyaluronic acid having an average molecular weight ranging from 200 to 750 KDs in amounts ranging from 0.1 and 40%.

40. The multilayer composite material according to claim 39, wherein said matrix consist of fibres of hyaluronic acid benzylester having an esterification degree of 75% in amounts ranging from 14 to 24%, demineralised bone matrix in amounts varying between 60 and 80%, hyaluronic acid having an average molecular weight ranging from 500 to 700 KDs in amounts comprised between between 5 and 10%.

41. The multilatyer composite material according to claim 1, wherein said inner matrix is immersed in a polymer to make the final matrix more compact and to fixable to the layer/s.

42. The multilayer composite according to claim 41 wherein said polymer is selected from:

- hyaluronic acid benzyl ester with a percentage of esterification of between 55 and 100%;
- fibrin glue,
- photocross-linkable polymers
- collagen and derivatives thereof.

43. The multilayer composite material according to claim 1 wherein the layer(s) comprise a hyaluronic acid ester.

44. The multilayer composite materials according to claim 43, wherein said hyaluronic acid is the benzylester with a percentage of esterification ranging between 50 and 100%.

45. The multilayer composite material according to claim 44, wherein said percentage degree is comprised between 75 and 100%.

46. The multilayer composite material according to anyone of claims 43-45, wherein the layers are in the form of: a non-woven material, containing fibres of the hyaluronic acid ester possibly associated with natural polymers selected from collagen and cellulose and the derivatives thereof, or synthetic polymers selected from poly-lactic acid, poly-glycolic acid and poly-caprolactone acid.

47. The multilayer composite material according to anyone of claims 43-45 wherein the layers are in the form of a woven material containing fibres of the hyaluronic acid ester, possibly subsequently immersed in a solution of hyaluronic acid.

48. The multilayer composite material according claim 1, wherein the layers are in the form of compact perforated porous or microporous membranes and films.

49. The multilayer composite materials according to anyone of claims 1-48, further containing pharmacologically and/or biologically active ingredients.

50. The multilayer composite materials according to claim 49, wherein the pharmacologically active ingredients are selected from the group consisting of antibiotics, antineoplastics, anti-inflammatories, cytokines, vitamins and cytotoxic, cytostatic and antiviral agents.

51. The multilayer composite materials according to claim 49, wherein biologically active ingredients contain trophic, osteoinductive, angiogenetic factors.

52. The multilayer composite material according to claim 51, wherein the trophic, osteoinductive and angiogenetic factors contain BMP, TGF, PDGF, FGF, EGF, IGF and VEGF.

53. The multilayer composite material according to anyone of claims 1-52 loaded with bone marrow cells.

54. The multilayer, composite material according to anyone of claims 1-51, loaded with autologous and/or allogenic mesenchymal cells either undifferentiated or partially differentiated into osteoblasts.

55. The multilayer composite materials according to anyone of claims 1-51, loaded with autologous and/or allogenic mesenchymal cells that are completely differentiated into osteoblasts.

56. A process for preparing the multilayer composite material according to anyone of claims 1-55 comprising the following steps:

a) forming the inner matrix by associating hyaluronic acid and/or a hylauronic acid ester and demineralised bone matrix, and/or a biocompatible biodegradable ceramics and/or bone of autologous or allogenic type or of animal origin,

b) coupling the matrix with the layer(s),

c) fixing the matrix to the layer(s), in toto or by means of the outer edge.

57. The process according to claim 56, wherein step (c) is carried out by heat treatment .

58. The process according to claim 56 wherein step (c) is carried out by exposing the material coming step (b) to a needle-punching process.

59. The process according to claim 58 wherein step (c) is carried out by sewing the material coming from step (b) with thread made of hyaluronic acid and/or the derivatives thereof or another biocompatible and bioresorbable polymer.

60.A bone substitute or graft consisting of the multilayer composite material according to anyone of claims 1-55.

62. A bone substitute or graft consisting of the multilayer composite material according to anyone of claims 18-56.

63. The bone substitute or graft according to claim 62 in the form of a sandwich or bag.

64. The bone substitute or graft according to anyone of claims 61-63 for use in the regeneration or formation of bone tissue.

65. The bone substitute or graft according to anyone of claims 61-63 for use in surgery.

66. The bone substitute or graft according to anyone of claims 61-65 for use in spinal surgery.

67. The bone substitute or graft according to anyone of claims 61-65 for use in spinal surgery.

68. The bone substitute or graft according to anyone of claims 61-65 for use in maxillofacial surgery.

69. The bone substitute or graft according to anyone of claims 61-65 for use in surgery to the shoulder, hand and foot.

70. The bone substitute or graft according to anyone of claims 61-65 for use in dental surgery.

71.The bone substitute or graft according to anyone of claims 61-65 for use in oncological surgery.

72. The bone substitute or graft according to anyone of claims 61-65 for use in all types of orthopaedic surgery requiring the fusion of adjacent bones and then the formation of new bone tissue.

73. The bone substitute or graft according to claim 67 for use in fusing to two adjacent vertebral bodies.

74. The bone substitute or graft according to claim 67 for use in filling one or more vertebral bodies previously hollowed out.